In The Name Of God

Title:

Anti Microbial Peptides

By:

Niloufar Tavakoli

Student of Qazvin university of medical sciences

With assistance:Dr.Ahmadpour

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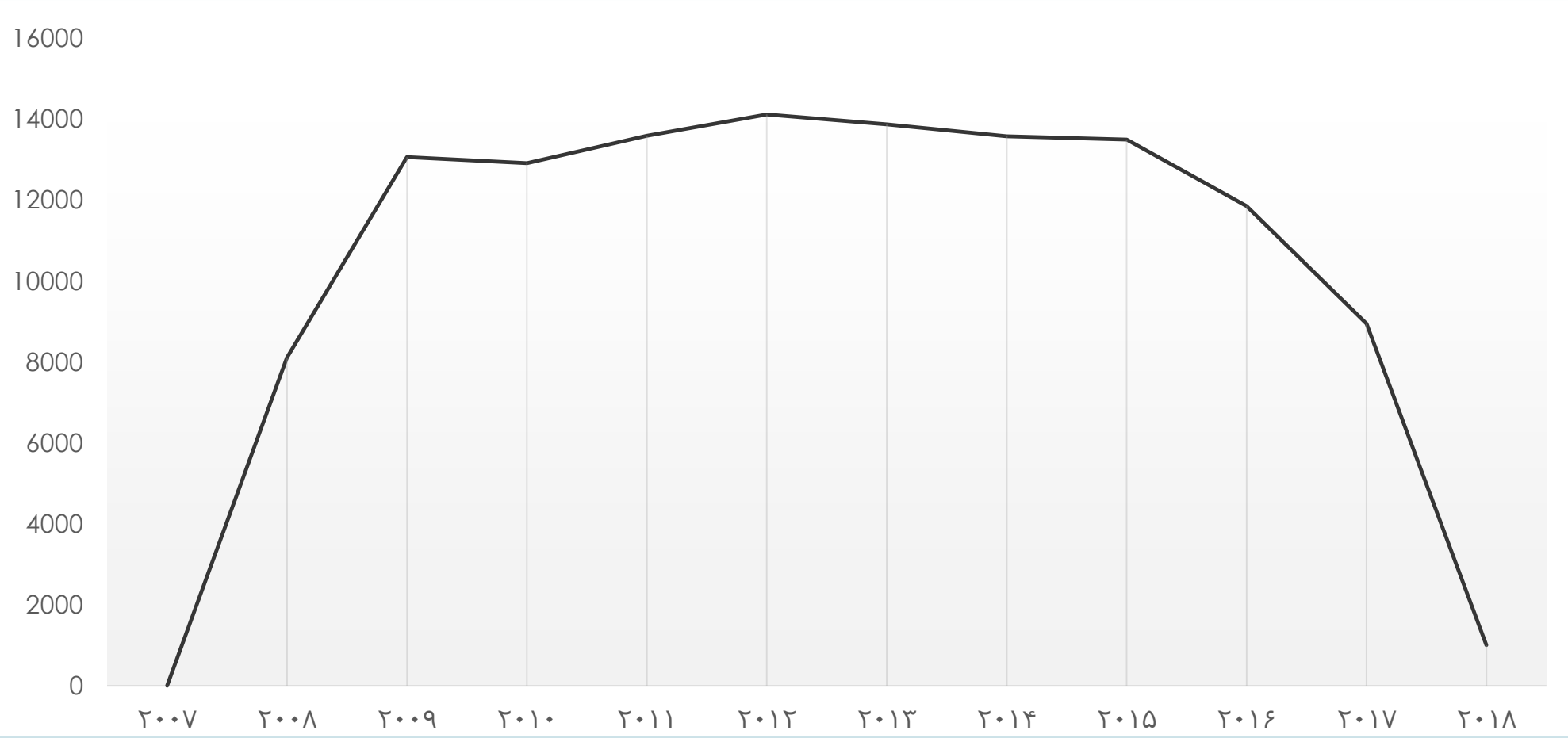
Chart:



PubMed-NCBI

Introduction:

Global concerns:

- the increased appearances of multi-resistant bacterial pathogens
- the rise in the incidence of cancer(6)

What are AMPs?

1) small peptides essential for the innate immune response
2) presenting activity against a wide range of pathogens

anticancer peptides (ACPs):

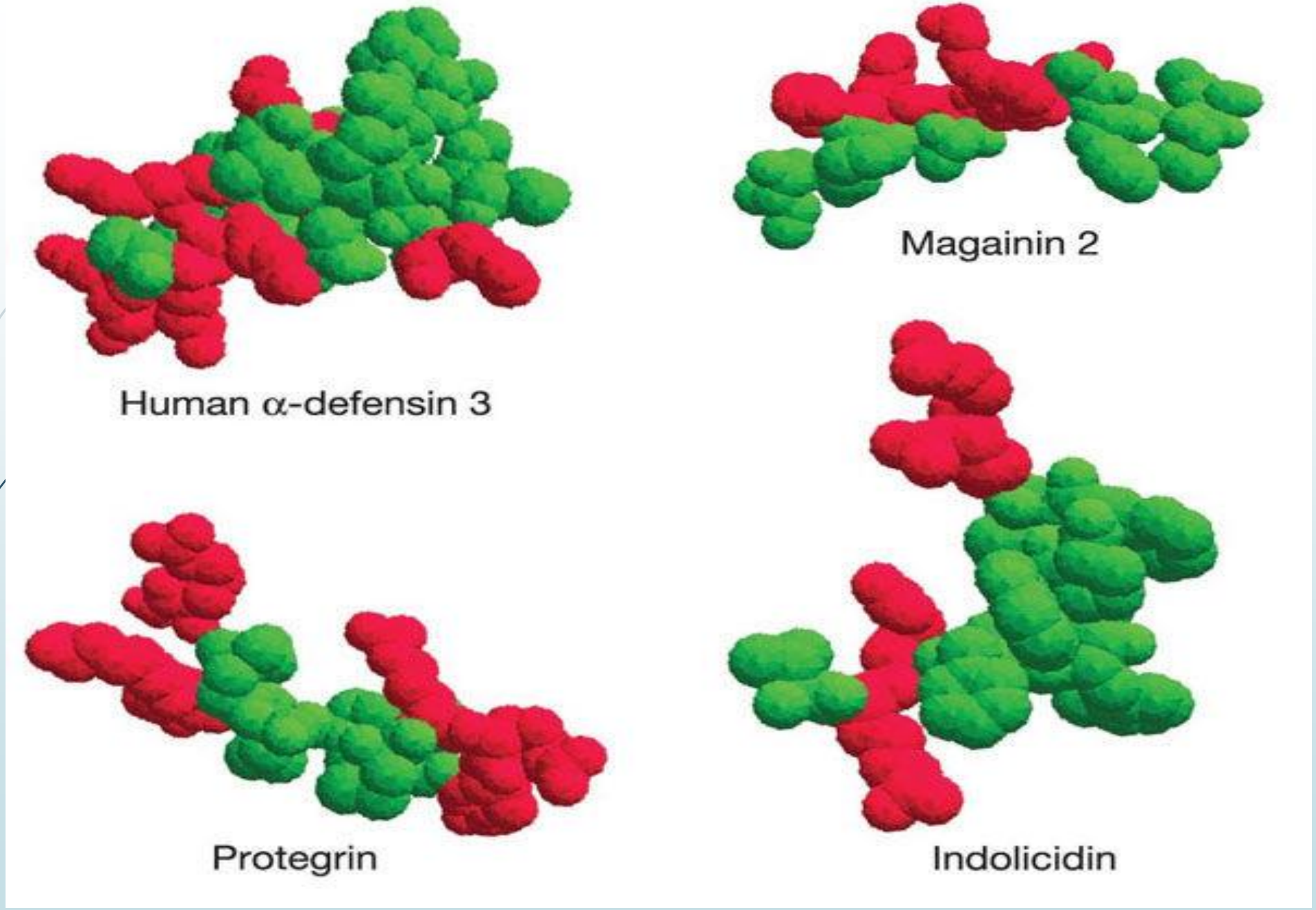
some of these peptides with anticancer activity(7)

What are AMPs?

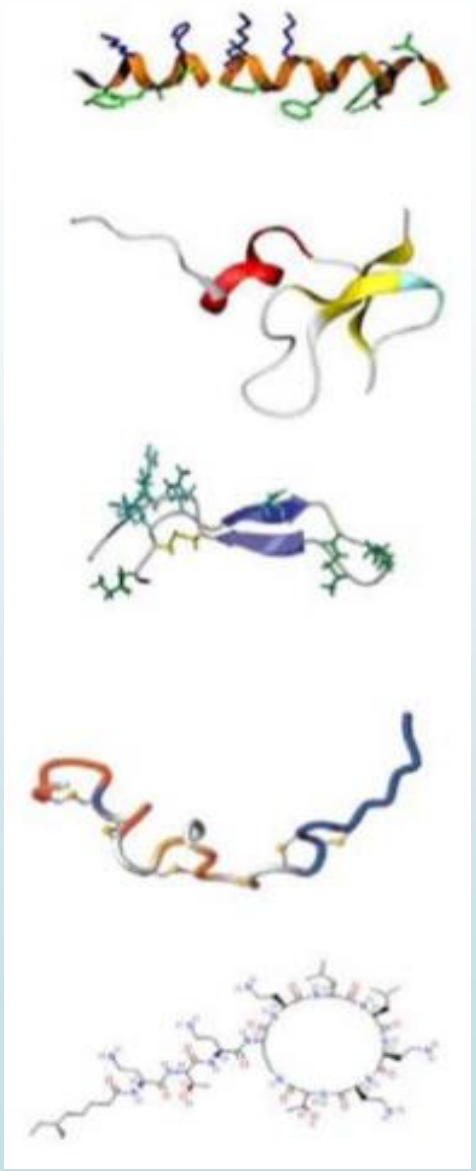
1) 5 – 40 amino acid residues

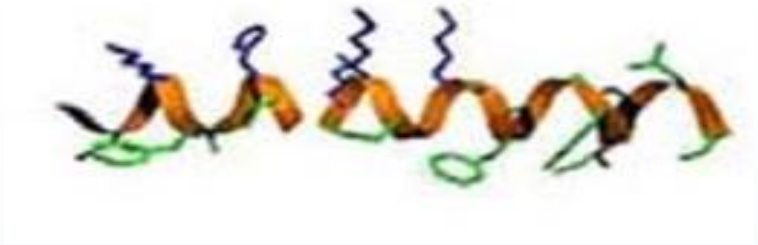
2) Exist in both eukaryotes and prokaryotes

3) mostly cationic (between +2 and +9 at neutral pH): imparted by the presence of multiple lysine and arginine residues (8)


Human α-defensin 3
Magainin 2
Protegrin
Indolicidin

Introduction of some AMPs:





Magainin2:(6)

Class: Alpha- helical
Linear peptide
Species: skin of the African frog Xenopus laevis
Activities:
1) Antimicrobial(Gram+ & Gram-)activity
2) Antifungi activity
3) Anti-endotoxin activity
4)Anti cancer activity: lyse hematopoietic tumor and solid tumor cells with little toxic effect on normal blood lymphocytes

Defensins:(6)

- 3-5 kDa cysteine-rich peptides
- found both in vertebrates and invertebrates
- mammalian defensins based on their size and pattern of disulfide bonding:

α-, β- and θ-defensins

- α- and β-defensins : found in neutrophils, epithelial cells, certain macrophage populations and Paneth cells of the small intestine

Beta-defensin 3:(6)

Class: all beta
Species: Homo sapiens
Activities:
1) Antimicrobial(Gram+ & Gram-)activity
2) act as chemoattractants for monocytes: stimulating their TNF and IL-1 expression
3) enhance the expression of IL8 in lung epithelial cells
4) exert chemotactic activity towards cells engaged in adaptive immunity response

LL-37:(6)

- Amphipathic, helical peptide
- Activities:

1) antimicrobial activity
2) regulating the inflammatory response
3) chemo-attracting cells of the adaptive immune system to wound or infection sites
4) stimulation of angiogenesis and chemotaxis of neutrophils, monocytes and T-cells

- Found in epithelial cells of testis, gastrointestinal and respiratory tract, in skin and in leukocytes

Lactoferricin B:(6)

Class: Beta-hairpin peptide
Species: Bos Taurus
Activity:
1)Antimicrobial(Gram+ & Gram-) activity
2)Anti Fungi activity
3)Anti virus activity
4)Anti cancer activity

Nisin A:(6)

Class: non- regular peptide
Species: Lactococcus lactis
Activity: Anti Gram+ activity

Polymyxin E(colistin):(6)

Class: Cyclic polypeptide
Species: Bacillus colistinus
Activity: Anti Gram – activity

Function of AMPs:

- recognize their target via electrostatic interactions

- engage with and disrupt microorganismal membranes(7)

Why AMPs selectively target microbial versus host membranes?(6)

Bacterial membranes are negatively charged(has phosphatidylglycerol (PG), cardiolipin (CL), or phosphatidylserine (PS).

On the contrary, mammalian membranes are enriched in zwitterionic phospholipids (has phosphatidylethanolamine (PE), phosphatidylcholine (PC), or sphingomyelin (SM).

the presence of cholesterol:

1) can reduce the activity of AMPs stabilizing the lipid bilayer
2) directly interacting and neutralizing them

Division based on function:

1. Anti microbial activity

2. Anti tumor activity(7)

3. Anti viral activity(6)

1. Anti microbial activity:(6)

1.1 Permeabilization mechanisms

1.2 Alternative mechanism of action: intracellular targets

1. Anti microbial activity:

Have strong interactions with electronegative charges on the bacterial surface

Such as:

- lipopolysaccharide (LPS) of gram-negative bacteria
- lipoteichoic acid (LTA) on gram-positive bacterial membranes(1)

Antimicrobial activity:

- For gram negative bacteria:

the AMP needs to permeabilize the outer membrane

- for gram positive bacteria:

the AMP only needs to diffuse through the peptidoglycan layer via nano-sized pores(1)

Antimicrobial activity:

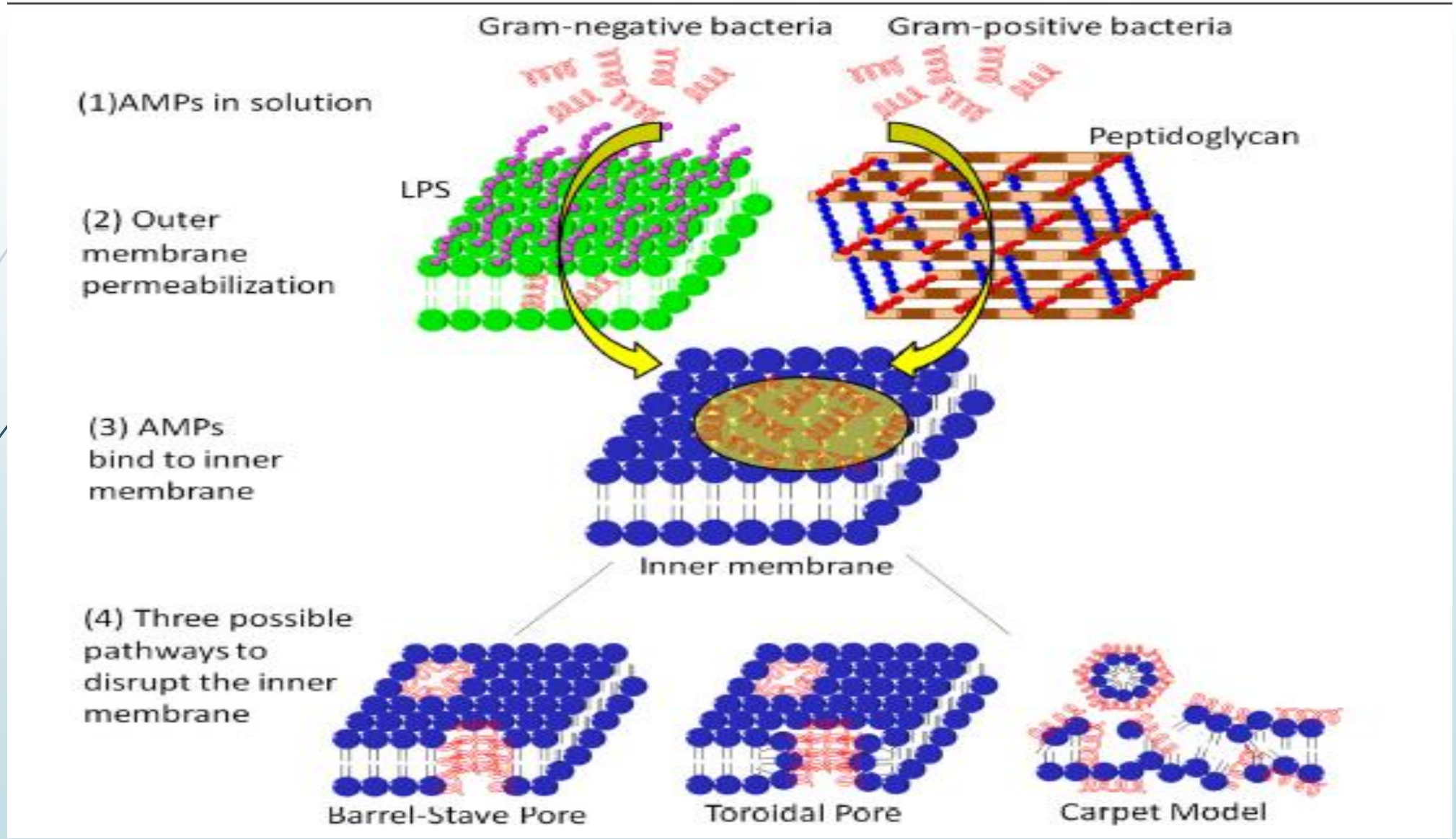
After adsorption , AMP can induce membrane pores such as:

1) Barrel-stave pore

2) Toroidal pore

3) Carpet model(1)

Anti microbial activity:(1)



Membrane pores:(3)

- result in the loss of membrane potential
- rapid release of intracellular components
- death

barrel-stave pores:

- the membrane does not display significant curvature and the hydration of the membrane remains unchanged
- both electrostatic and hydrophobic interactions are important since the AMP molecules interact with both the head groups and the lipid tails

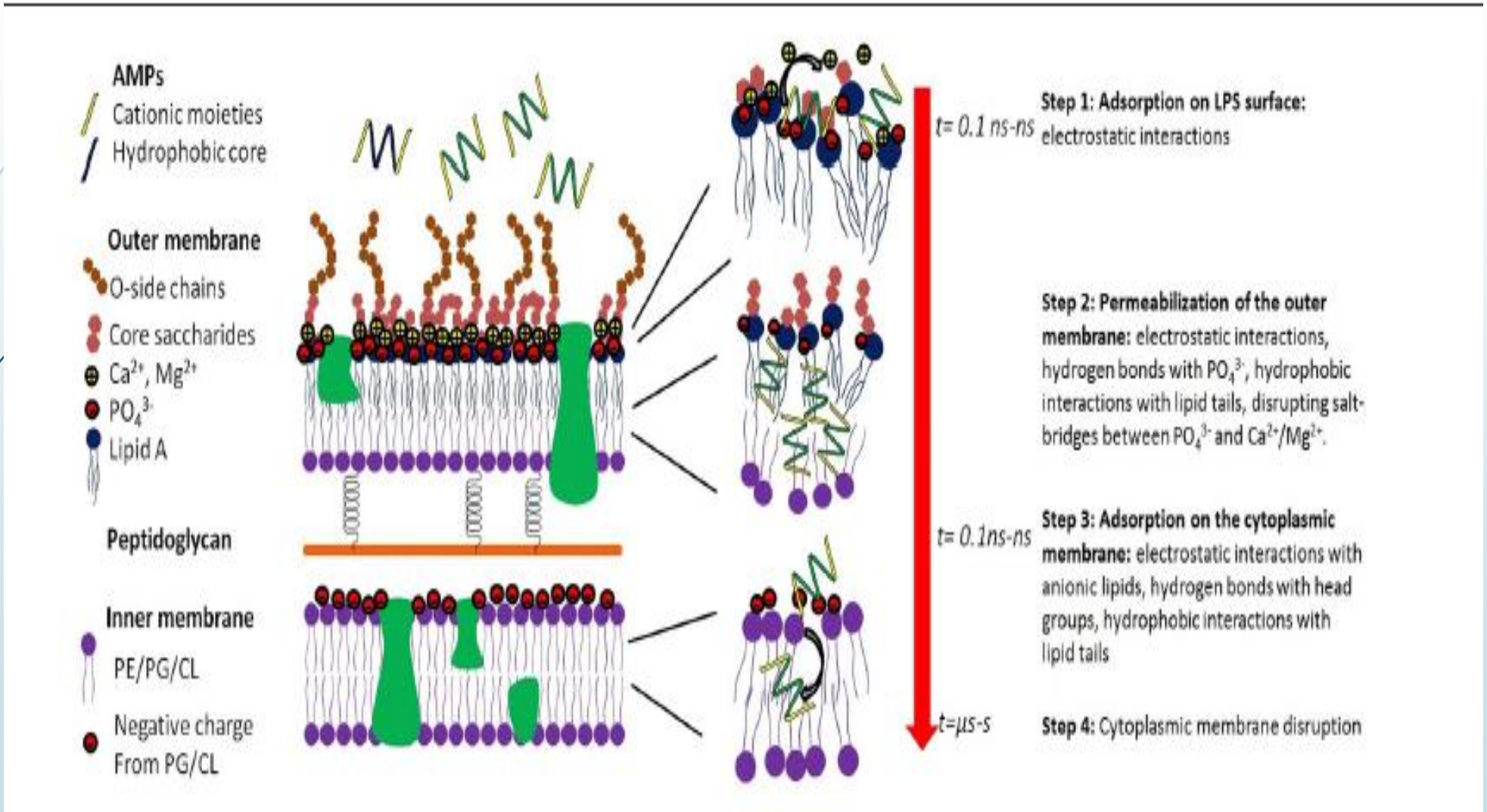
toroidal pores:

- the AMP molecules penetrate deeper into the membrane
- the head groups of the lipids are dragged into the lipid tail
- The lipid tails are packed away from the surface of the pore
- AMPs primarily interact with the pores electrostatically since the surface of toroidal pores are covered by the phosphate head groups and the AMP molecules have less hydrophobic contact with the lipid tails

Carpet model:

- AMPs adsorb onto the membrane surface
- orient parallel to the membrane
- As their surface concentrations reach a critical value, these AMPs disrupt the integrity of the membrane
- this process called carpet mechanism or detergent model

Anti microbial activity:(1)



Alternative mechanism of action: intracellular targets:

- Indolicidin: inhibit DNA synthesis in Escherichia coli
- Attacins: block the transcription of the omp gene in E. coli
- magainins and cecropins: induce selective transcription of stress-related genes micF and osmY in E.coli
- PR-39: kill bacteria by blocking both DNA and protein synthesis(6)

2)Anti tumor activity:

factors contribute to elevated negative charges on cancer cells:

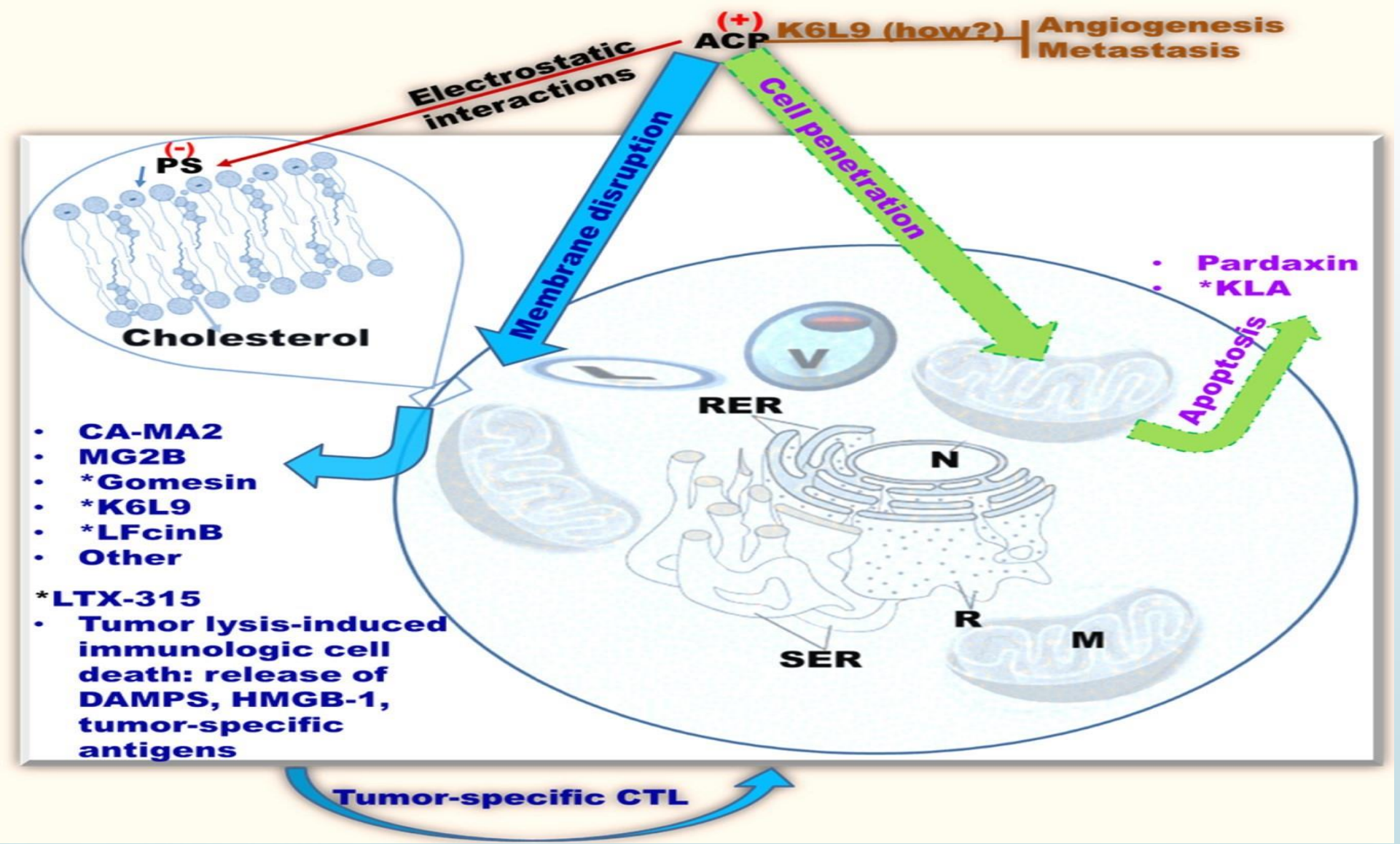
- phosphatidylserine in the outer leaflet of membrane
- heparin sulfates
- O-glycosylated mucins on the surface of tumor cells(2)

Anticancer peptides(ACPs)modes of action:

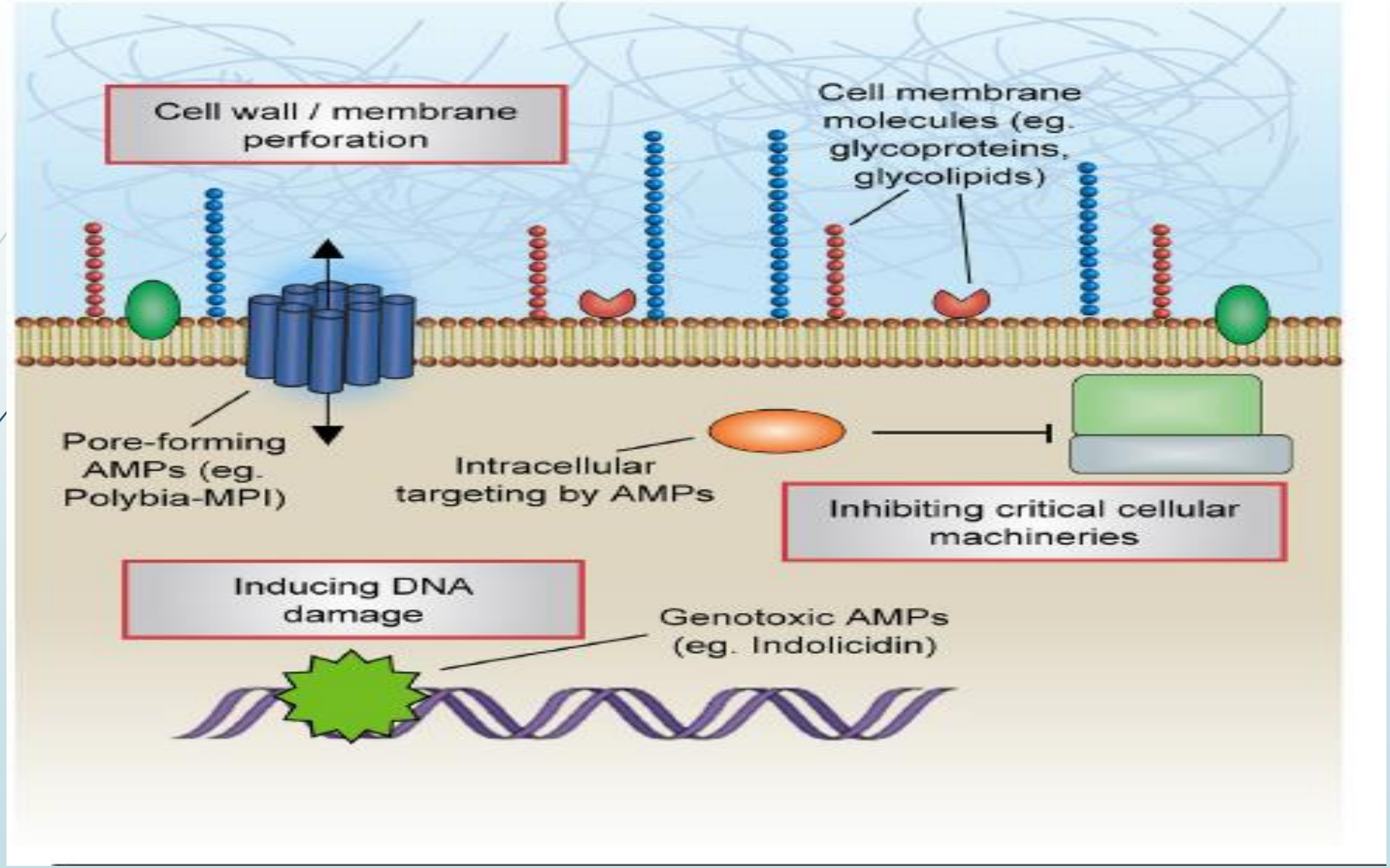
1. disruption of plasma/ mitochondrial membranes
2.necrosis
3.apoptosis
4.mechanisms of mediated immunity
5.membrane receptors involvement
6.inhibition of DNA synthesis
7.anti-angiogenic effects

Different ACPs can act by more than one mechanism simultaneously .(2)

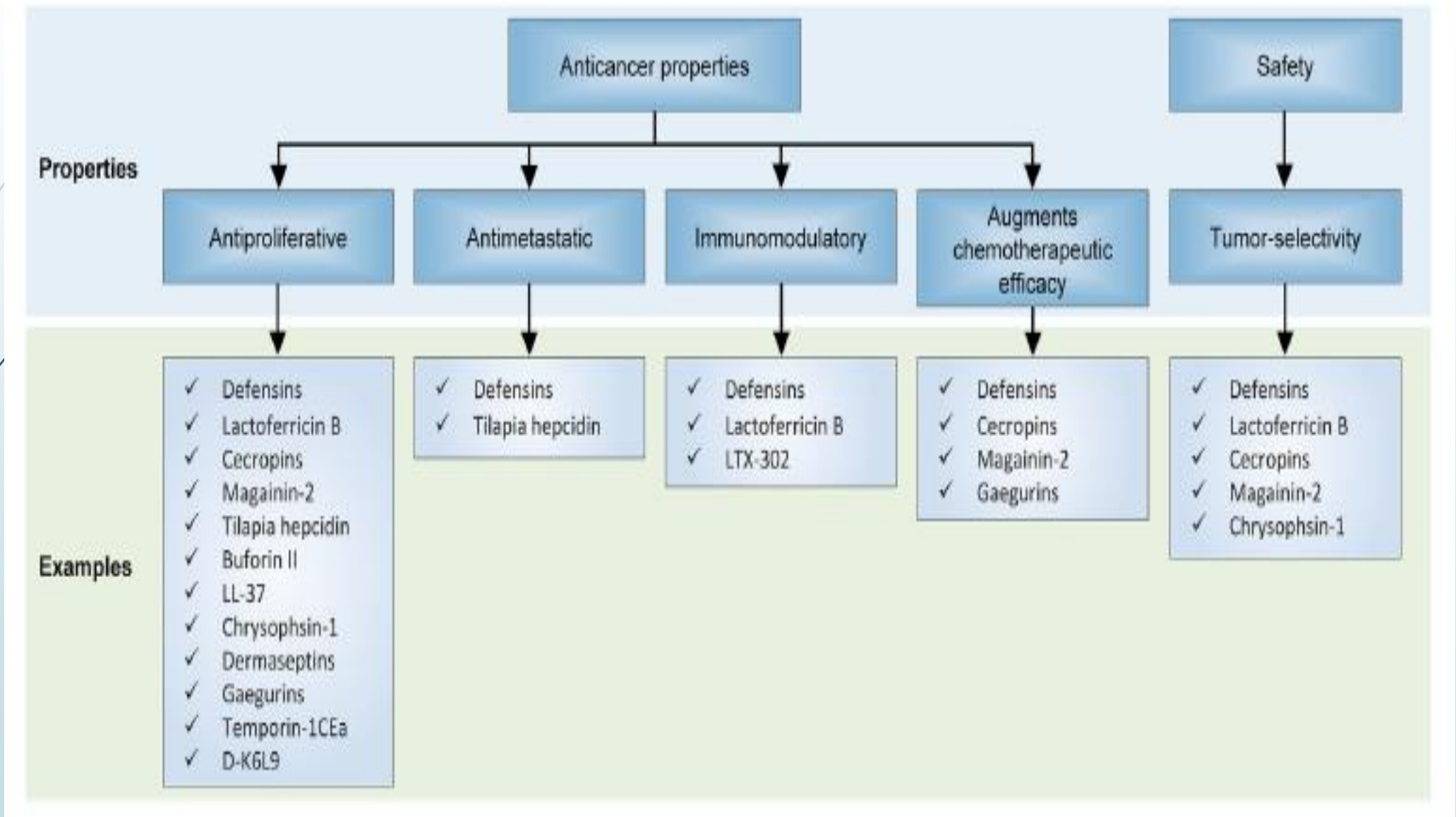
Anti cancer activity:(2)



Anti cancer activity:(3)



Anti cancer activity:(3)



Anti viral activity:(6)

- Certain AMPs have been shown to inhibit the replication of enveloped viruses such as influenza A virus , vesicular stomatitis virus (VSV) and human immunodeficiency virus (HIV-1)
- mechanism of antiviral action:

direct interaction of these peptides with the envelope of the virus, leading to permeation of the envelope and, eventually, lysis of the virus particle.

Division based on structure:

diverse in both amino acid compositions and secondary structures:

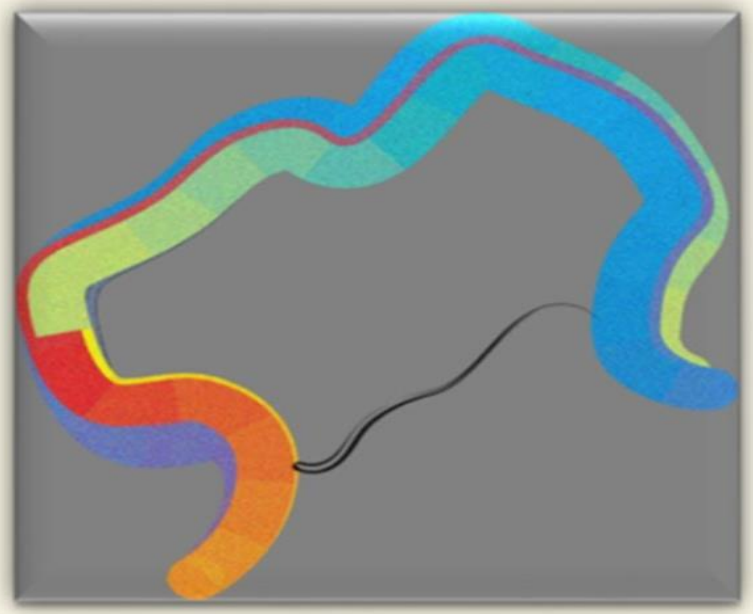
- α-helix
- β-sheets
- extended helix
- and loop(2)

Main structural classes of cationic antimicrobial peptides(AMPs):(2)

α-helices (*e.g.*, cathelicidins, magainins)

β-sheets (*e.g.*, defensins, thionins)

Loop (*e.g.*, bactenicin)



Other:

- extended helices (*e.g.*, indolicidin)
- Cyclic (*e.g.*, cyclotides, retrocyclin)
- mixed structures (*e.g.*, protegrins)

Division based on source:(2)

AMP name	Amino acid sequence	Source
Alpha-defensin-1	ACYCRIPACIAGERRYGTCI YQGRLWAFCC	Human
BMAP-28	GGLRSLGRKILRAWKKYG	Bovine
Brevenin-2R	KFALGKVNAKLQSLNAKSLK QSGCC	Frog
Gomesin	ZCRRLCYKQRCVTYCRGR	Spider
Pardaxin	GFFALIPKIISSPLFKTLLSAVGS ALSSSGGQE	Fish

Amp name	Amino acid sequence	Source
CA-MA-2	KWKLFKKI-P-KFLHSAKKF	Hybrid
chrysophsin-1	FFGWLIKGAIHAGKAIHGLI	Red sea bream
D-K6L9	LKLLKKLLKKLLKLL	Engineered
LL37	LLGDFFRKSKEKIGKEFKRIV QRIKDFLRNLVPRTES	Human
Melittin	GIGAVLKVLTTGLPALISWIK RKRQQ	Insect

Division based on tumor target:(2)

AMP name	Amino acid sequence	Tumor target
Alpha-defensin-1	ACYCRIPACIAGERRYGTCIY QGRLWAFCC	HTC/STC
Brevenin-2R	KFALGKVNAKLQSLNAKSLK QSGCC	STC
chrysophsin-1	FFGWLIKGAIHAGKAIHGLI	HTC/STC
KLA	RRQRRTSKLMKRGGKLAKL-AKKLAKLAK- (KLAKLAK)2	STC

AMP name	Amino acid sequence	tumor target
BMAP-28	GGLRSLGRKILRAWKKYG	HTC
CA-MA-2	KWKLFKKI-P-KFLHSAKKF	STC
D-K6L9	LKLLKKLLKKLLKLL	STC
pardaxin	GFFALIPKIISSPLFKTLLSAVG SALSSSGGQE	STC

Antimicrobial peptides for oncologic indications in ongoing clinical trials:(3)

phase	Peptide name	condition	Administration route
I	LL-37	Metastatic melanoma	Intratumoral
I	LTX-315	Solid tumors	Intravenous
II	ANG-1005	Breast and brain metastasis	Intravenous
III	ITK-1	Glioblastoma and prostate cancer	Intravenous

Challenges:

- LL-37:promotes ovarian tumor cell proliferation by over-expression(4)

[Mechanisms of antimicrobial peptide LL-37 in macrophage-promoted ovarian cancer cell proliferation].

[Article in Chinese]
Li D[1], Wang X, Dai Y, Yang F, Wan HY[2].

Author information

Abstract

OBJECTIVE: The aim of this study was to investigate the role of macrophages in promotion of ovarian tumor cell proliferation mediated by over-expression of antimicrobial peptide LL-37.

METHODS: To co-culture ovarian tumor cells SKOV3, 3AO and HO-8910 with macrophages. The Transwell(®) inserts system was used in the co-culture model. The effect of macrophages promoted ovarian tumor cell proliferation was assessed by BrdU-ELISA and cell number counting. Expressions of mRNA and protein of LL-37 in the macrophages and SKOV3 cells were determined by RT-PCR and Western blot analysis. To observe that LL-37 is responsible for macrophage-promoted ovarian tumor cells growth, LL-37 neutralizing antibody was added to abrogate the LL-37 activation.

RESULTS: The cell number assay showed that after 4 days coincubation with macrophages in the proportion of 1:0.5, the number of SKOV3 cells increased from (6.0 ± 0.5)×10(4) to (11.8 ± 1.3)×10(4), showing a significant difference ($P < 0.05$). It also showed that the growth of the SKOV3 cells was dependent on the macrophage number ($P < 0.05$). The number variability of 3AO and HO-8910 cells was as the same as SKOV3 cells upon co-culture with macrophages. As determined by BrdU-ELISA, the resulted proliferation of ovarian tumor cells was similar to the result of cell number counting. RT-PCR and Western blot results showed that the expression of LL-37 mRNA and protein in the macrophages was remarkably enhanced in a time dependent manner upon coincubation with SKOV3 cells, but did not work in SKOV3 cells. BrdU-ELISA assay exhibited that treatment of cells with LL-37 significantly stimulated HO-8910 and 3AO cell proliferation. Addition of LL-37 neutralizing antibody markedly inhibited macrophage-promoted ovarian tumor cell (SKOV3, 3AO and HO-8910 cells) proliferation. The OD values of these three cells were decreased from 2.95 ± 0.11 to 1.45 ± 0.04, from 3.39 ± 0.36 to 1.32 ± 0.09 and from 3.93 ± 0.17 to 1.68 ± 0.23, respectively ($P < 0.05$).

CONCLUSIONS: Over-expression and release of LL-37 from macrophages is responsible for proliferation of ovarian tumor cells in co-culture condition. The data presented indicate that LL-37 may be critical for macrophage-induced tumor progression.

Challenges:

- Cathelicidin: promotes colon cancer by activating the wnt/B-catenin pathway(5)

Cathelicidin, an antimicrobial peptide produced by macrophages, promotes colon cancer by activating the Wnt/β-catenin pathway

Dong Li[1,*], Wenfang Liu[2,*], Xuan Wang[3], Junlu Wu[1], Wenqiang Quan[1], Yiwen Yao[1], Robert Bals[4], Shurong Ji[2], Kaiyin Wu[5], Jia Guo[6], Haiying Wan[1]

[1]Department of Clinical Laboratory, Tongji Hospital of Tongji University, 200065 Shanghai, China

[2]Department of General Surgery, Tongji Hospital of Tongji University, 200065 Shanghai, China

[3]Department of Pharmacy, Putuo People's Hospital, 200060 Shanghai, China

[4]Department of Internal Medicine V – Pulmonology, Allergology, Respiratory Intensive Care Medicine, Saarland University Hospital, 66424 Homburg, Germany

[5]Institute of Pathology, Charité University Hospital, 12200 Berlin, Germany

[6]Tongji University Suzhou Institute, 215000 Suzhou, China

*These authors have contributed equally to this work

Correspondence to:
Dong Li, **e-mail:** 186ld@163.com

Keywords: Cathelicidin, Colon cancer, Macrophage, Wnt/β-catenin, Growth

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ABSTRACT

Here we found that levels of cathelicidin, an antimicrobial peptide, were increased in colon cancer tissues compared to noncancerous tissues. Importantly, cathelicidin was mainly expressed in immune cells. Contact with tumor cells caused macrophages to secrete cathelicidin. Neutralization of cathelicidin, *in vivo*, significantly reduced the engraftment of macrophages into colon tumors, as well as proliferation of tumor cells, resulting in an inhibition of tumor growth. Furthermore, treatment with cathelicidin neutralizing antibody de-activated the Wnt/β-catenin signaling pathway in tumor cells both *in vivo* and *in vitro*. Cathelicidin activated Wnt/β-catenin signaling by inducing phosphorylation of PTEN, leading to activation of PI3K/Akt signaling and subsequent phosphorylation of GSK3β, resulting in stabilization and nuclear translocation of β-catenin. These data indicate that cathelicidin, expressed by immune cells in the tumor microenvironment, promotes colon cancer growth through activation of the PTEN/PI3K/Akt and Wnt/β-catenin signaling pathways.

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